

FIGURE 1

The Universal Code Equivalent of  
the Mitochondrial 1-Sce I Gene.

AAAAAATAAATCAT ATG AAA AAT ATT AAA AAA AAT CAA GTC ATG AAT CTC CGT CCT AAT TCT  
M K N I K K N Q V M N L S P H S  
AAA TTA TTA AAA GAA TAT AAA TCA CAA TTA ATT GAA TTA AAT ATT GAA CAA TTT GAA GAA  
K L L K E Y K S Q L I E L N I E Q F E A  
GGT ATT GGT TTA ATT TTA GGA GAT GCT TAT ATT COT ACT GGT GAT GAA GGT AAA ACT TAY  
G I G L I L G D A Y I R S R O E S K T Y  
TGT ATG CAA TTT CAC TCC AAA AAT AAG CCA TAC ATG GAT CAT GTC TGT TTA TTA TAT GAT  
C M Q F E W K N K A Y M O H V C L - Y D  
CAA TGG GTA TTA TCA CCT CCT CAT AAA AAA GAA AGA GTT AAT CAT TTA GGT AAT TTA GTA  
Q W V L S P M H K K E R V M H L Q N - V  
ATT ACC TGG GGA GCT CAA ACT TTT AAA CAT CAA GCT TTT AAT AAA TTA GCT AAC TTA TTT  
I T W G A Q T F K H Q A F M K L A N L F  
ATT GTC AAT AAT AAA AAA CTT ATT CCT AAT AAT TTA GTT GAA AAT TAT TTA ACA CCT ATG  
I Y N H K K L I P H N L V E N Y L T P H  
AGT CTG GCA TAT TGG TTT ATG GAT GAT GGA GGT AAA TGG GAT TAT AAT AAA AAT TGT CCT  
S L A Y W F M D O Q G K W D Y N K H S L  
AAT AAA AGT ATT GTC TTA AAT ACA CAA AGT TTT ACT TTT GAA GAA GTC GAA TAT TTA CCT  
N K S I V L N T Q S F T F E E V C Y L V  
AAA GGT TTA AGA AAT AAA TTT CAA TTA AAT TGT TAT GTT AAA ATT AAT AAA AAT AAA CCA  
K G L R M K F Q L N C Y V K I N K N K P  
ATT ATT TAT ATT GAT TCT ATG AGT TAT CTG ATT TTT TAT AAT TTA ATT AAA CCT TAT TTA  
I I Y I D S M S Y L I F Y N I T K P Y L  
ATT CCT CAA ATG ATG TAT AAA CTG CCT AAT ACT ATT TCA TCC GAA ACT TTT TTA AAA TAA  
I P Q M H Y K L P N T I S S E T F L K

FIGURE 2

## The synthetic I-Sce I gene

Bam HI

CCGGATCCATCAT ATG AAA AAC ATC AAA AAA AAC CAG GTA ATG AAC CTG GGT CCG AAC TCT  
 M H M K H I K K N Q V M N L S P N S

AAA CTG CTG AAA GAA TAC AAA TCC CAG CTG ATC GAA CTG AAC ATC GAA CAG TTC GAA GCA  
 K L L K E Y K S Q L I E L N I E S F E A

1. GGT ATC GGT CTG ATC CTG GGT GAT GCT TAC ATC CQT TCT CGT GAT GAA GGT AAA ACC TAC  
 G I G L I L G D A Y I R S R D E G K T Y

TGT ATG CAG TTC GAG TCR AAA AAC AAA GCA TAC ATG GAC CAC GTA TGT CTG CTG TAC GAT  
 C M Q F E W K N K A Y M D H V C L L Y D

CAG TCC GTA CTG TCC CCG CCG CAC AAA AAA GAA CQT GTT AAC CAC CTG GGT AAC CTG GTA  
 Q W V L S P P H K K E R V N H L Q N L V

ATC ACC TGG GGC GGC CAG ACT TTC AAA CAC CAA GCT TTC AAC AAA CTG GCT AAC CTG TTC  
 I T W G A Q T F K M Q A F N K L A N L F

ATC GTT AAC AAC AAA AAA ACC ATC CCG AAC AAC CTG GTT GAA AAC TAC CTG ACC CCG ATG  
 I V N N K K T I P N N L V E H Y L T P M

2. TCT CTG GCA TAC TGG TTC ATG GAT GAT GGT GGT AAA TGG GAT TAC AAC AAA AAC TCT ACC  
 S L A Y W F M D O D S G X W D Y H K N S T

AAC AAA TCG ATC GTA CTG AAC ACC CAG TCT TTC ACT TTC GAA GAA GTA GAA TAC CTG GTT  
 N K S I V L N T O S F T F E E V E Y - V

AAG GGT CTG CGT AAC AAA TTC CAA CTG AAC TGT TAC GTA AAA ATC AAC AAA AAC AAA CCG  
 K G L R N K F Q L N C Y Y K I H K N K P

ATC ATC TAC ATC GAT TCT ATG TCT TAC CTG ATC TTC TAC AAC CTG ATC AAA CCG TAC CTG  
 I I Y I D S M S Y L I F Y N L I K P Y L

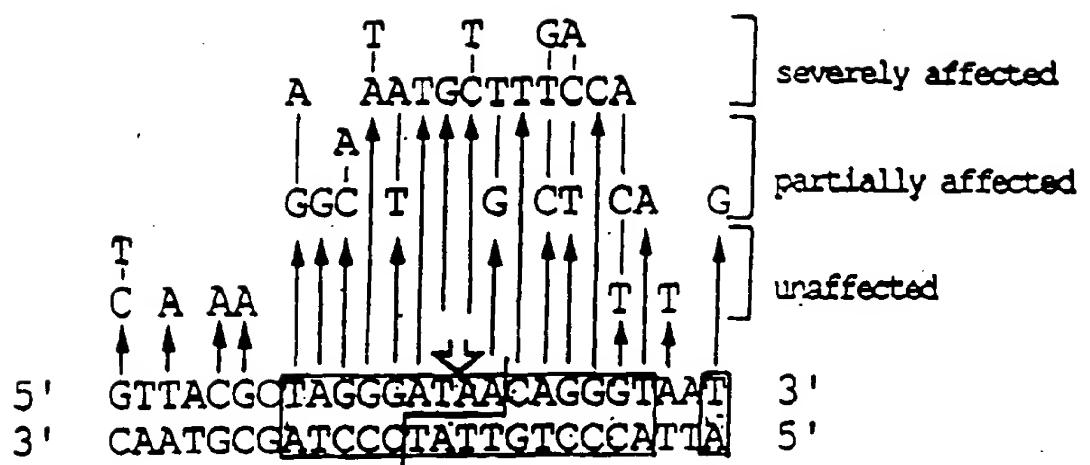
ATC CCG CAG ATG ATG TAC AAA CTG CCG AAC ACT ATC TCC TCC GAA ACT TIC CTG AAA TAA  
 I P Q M H Y K L Y N T I S S E T F L K

TAAGTCGACTCCAGGATCCGGTAAGTAAGTAA

Sal I Pst I Bam HI

1 and 2: These amino acids are absolutely necessary to produce catalytic activity. Other substitutions are possible, such as deletions of the 10 first amino acids.

FIGURE 3



**FIGURE . 4**

I-SceI coding sequence of pSCM525 - Note the two amino acid N-terminal extension as compared to genuine version of the gene.

FIGURE 5

VARIATIONS AROUND THE 1-Sce 1 SEQUENCE

-2	-1	1	5	10	20	30	40	50	60	70	80	90	100	110	120	130	140	150	160	170	180	190	200	210	220	230
H	H	Y	K	Y	K	N	I	E	L	N	I	E	S	P	R	Y	S									
K	L	I	X	E	T	K	S	O	L	I	E	L	N	I	E	S	F	E	A							
G	I	S	L	L	A	2	A	Y	I	R	S	R	D	E	S	K	-	I								
C	H	Q	F	E	W	K	N	X	A	Y	H	O	H	V	C	L	-	C								
G	W	Y	b	S	P	P	H	K	K	E	R	Y	N	M	L	G	N	-								
I	T	V	W	G	A	O	T	F	K	H	O	A	F	N	X	L	A	N	-							
I	V	N	N	K	K	I	I	P	N	N	L	V	E	H	Y	L	T	P	-							
S	L	A	T	W	R	A	2	2	2	2	2	K	W	D	Y	N	X	N	S	-						
N	K	S	I	V	L	N	T	O	S	F	T	F	E	E	V	E	Y	-	V							
K	G	L	R	N	K	F	O	L	N	C	Y	V	K	I	N	K	N	C	-							
I	I	Y	I	D	6	M	S	Y	L	I	F	Y	N	L	I	K	P	Y	-							
I	P	A	M	R	Y	K	L	P	N	T	I	S	S	E	T	F	L	K	-							

Positions that can be changed without affecting enzyme activity (demonstrated)  
positions -1 and -3 are not natural. The two amino acids are added due to cloning strategies  
positions 1 to 10: can be deleted  
position 36: G is tolerated  
position 40: M or Y are tolerated  
position 41: S or N are tolerated  
position 43: A is tolerated  
position 44: Y or N are tolerated  
position 91: A is tolerated  
positions 123 and 159: L are tolerated  
position 225: A and S are tolerated

Changes that effect enzyme activity (demonstrated)  
position 13: L to S  
position 38: I to S or N  
position 39: G to D or R  
position 40: L to Q  
position 42: L to R  
position 44: D to E, Q or H  
position 45: A to E or D  
position 46: Y to D  
position 47: I to R or Y  
position 80: L to S  
position 144: D to E  
position 145: Q to E  
position 146: Q to E

FIGURE 5

## Group I Intron Encoded Endonucleases and Related Endonucleases

Endonuclease	Recognition Sequence	Cleavage Site	Intron site
<b>Tus-Dodecapeptide Family (or the cutters)</b>			
I-Sce I ( <i>Saccharomyces</i> mitochondria)	CGCTAGGGATAAACAGGGAAATATACG GGCAAGCCCATTTTGTCCATTATTCGG		
I-Sce IV ( <i>Saccharomyces</i> mitochondria)	TTCTCATGATCAGCTCTTAACCCATGG AAGAGTACTAAUUGAGATTAGGACCC		
I-Sce II ( <i>Saccharomyces</i> mitochondria)	CTTGTCATCCAGAGAGTATATCTT GAACCGTAAAGCTTCTTCTTCTTCTT		
I-Cre I ( <i>Chlamydomonas</i> chloroplast)	TCACGGTCCAAAGGTTAGCCAAATCTA ATTCGCCAGGATTCCATCGCTTTAATC		
I-Hpo I ( <i>Physarum</i> nucleus)	TGACTCTCTAAAGGTAGCCAAATGGG ACTGAGAGAACTCCATTTCTTCTTCTT		
I-Sce III ( <i>Saccharomyces</i> mitochondria)	CGAGGTTTCTGCTAATCTATTATACG CCCTCCAAAUCATTGATCAAATTAATGG		
I-Cre I ( <i>Chlamydomonas</i> chloroplast)	GGGTTCAAAACGGTCTGGTAGAGACAGTT CCCAAGTTTCTGCTACACCTCTGCTCTA		
Endo Sce I (RF3) ( <i>Saccharomyces</i> mitochondria) Non-motile	GATGGCTGAGGCATAGGGTTGGTCAAG CTACGACATTCGGTAXCCGAACCAAATA		
HO ( <i>Saccharomyces</i> nucleus) Non-motile	CTTTCGCGAACAGTATAATTTTATAA CTTTCGCGAACAGTATAATTTTATAA		
I-Cam I ( <i>Chlamydomonas</i> mitochondria) (Putative endonuclease)	ACCATGGGGTCRAATGTTCTGGG TGGTACCCCAAGTTTACAGAAAGACCC		
I-Pan I ( <i>Podospora</i> mitochondria) (Putative endonuclease)	GTCGCTGAACTGATTTTTTCTTT CACCCACCTACCTATAATAAGGAAA		
<b>Bacteriophage T4</b>			
I-Tev I	GAACGCGCGAGATCTTCTGGCTCTTAA GTCGCGAGTGTAGAAAGAACCCAGATGGCGAAATTAA		
I-Tev II	CAAGCTTATGAGCTATGAACTGAAACACGTTATT GTCGAAATACCTACACTTCACCTGGCGAAATAA		
I-Tev III	GCTATTCGGTTTATGATCTTTGGCTCTTAA CCATGACCGAAATACATAGAAACCCACAGGGAAATTAA		

FIGURE 7

0544066277 054200

EXPRESSION VECTORS

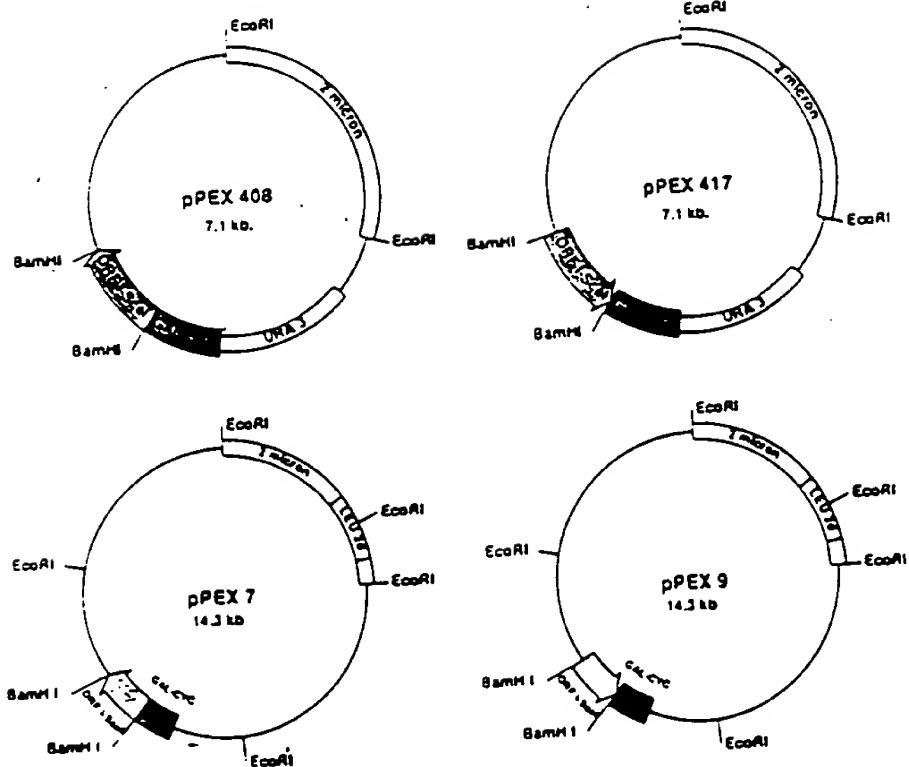
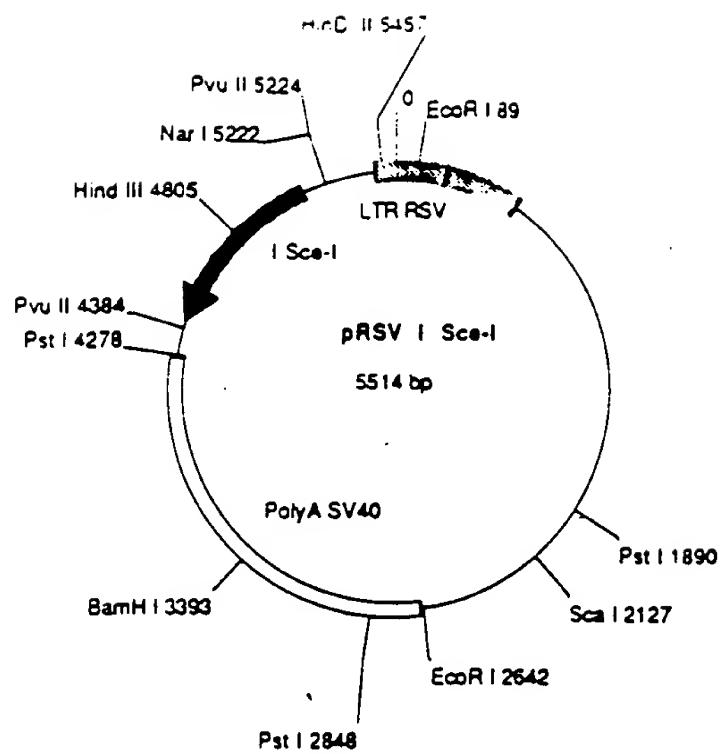


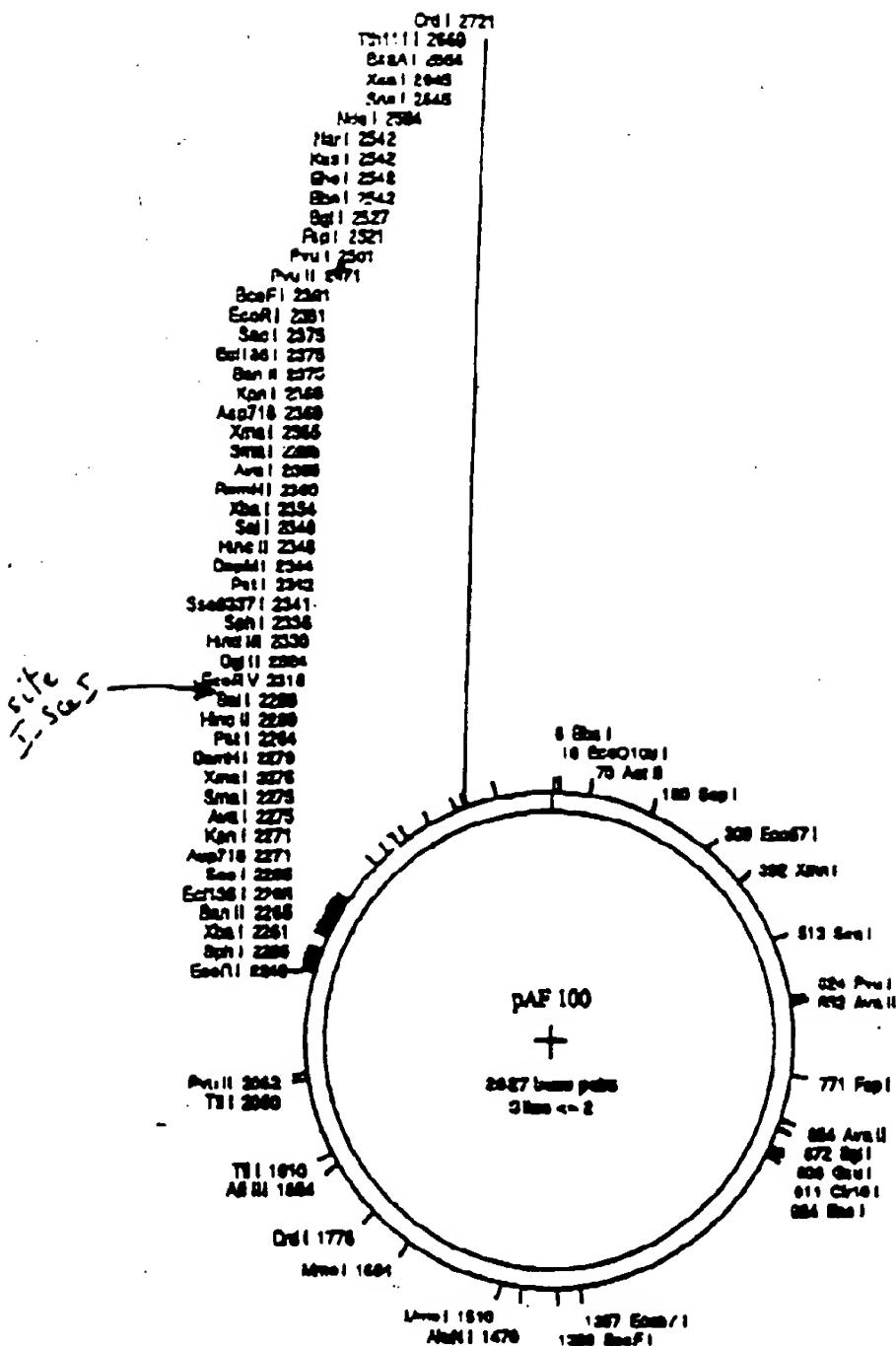
FIGURE 8



00000000000000000000000000000000

FIGURE 9

0 9 9 2 2 9 7 0 0 1 2 2 7 0 0



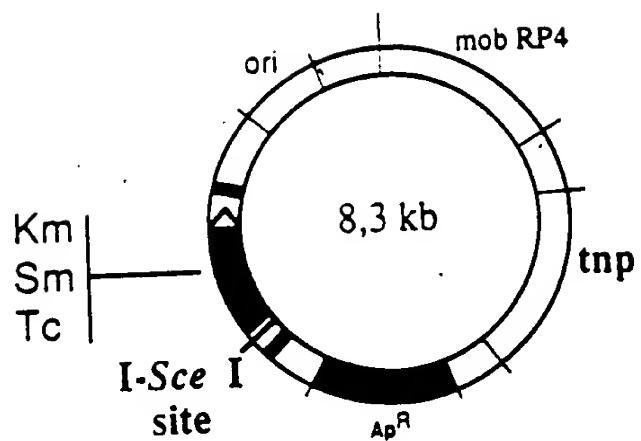
pAF 100 - RESTRICTION MAP

FIGURE 193

DAF 100 - RESTRICTION MAP

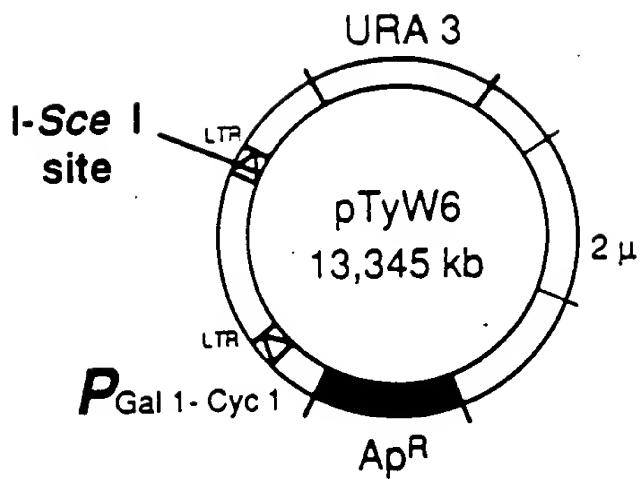
FIGURE 11

Names : pTSm ω  
pTKm ω  
pTTc ω



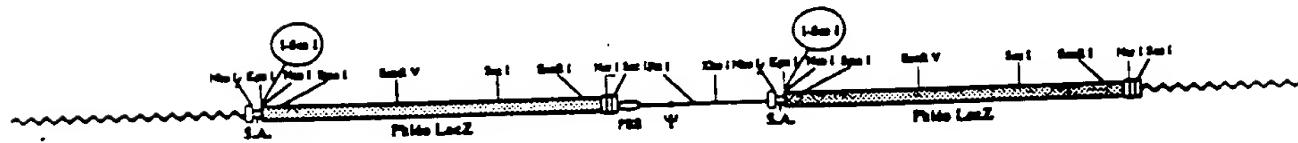
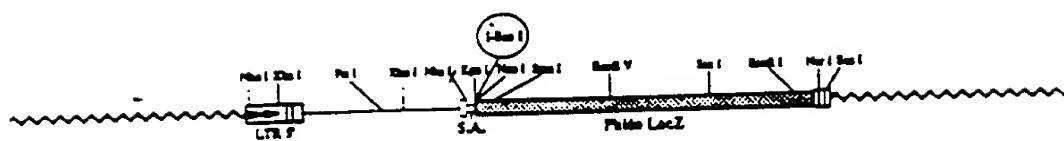
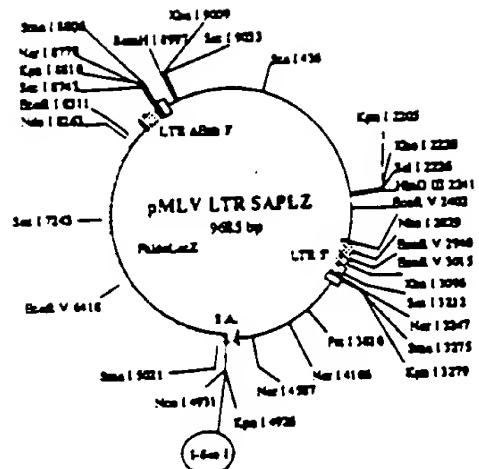
Construction : pGP 704 from De Lorenzo, with transposase gene and insertion of the linker [I-SceI] in NotI unique site

FIGURE 12



Construction : pD 123 , from J. D. Boeke  
with insertion of a linker [ I-SceI - NotI ] in BamHI

FIGURE 13



0.025% PIPES 0.025% SDS 0.1M EGTA

FIGURE 14

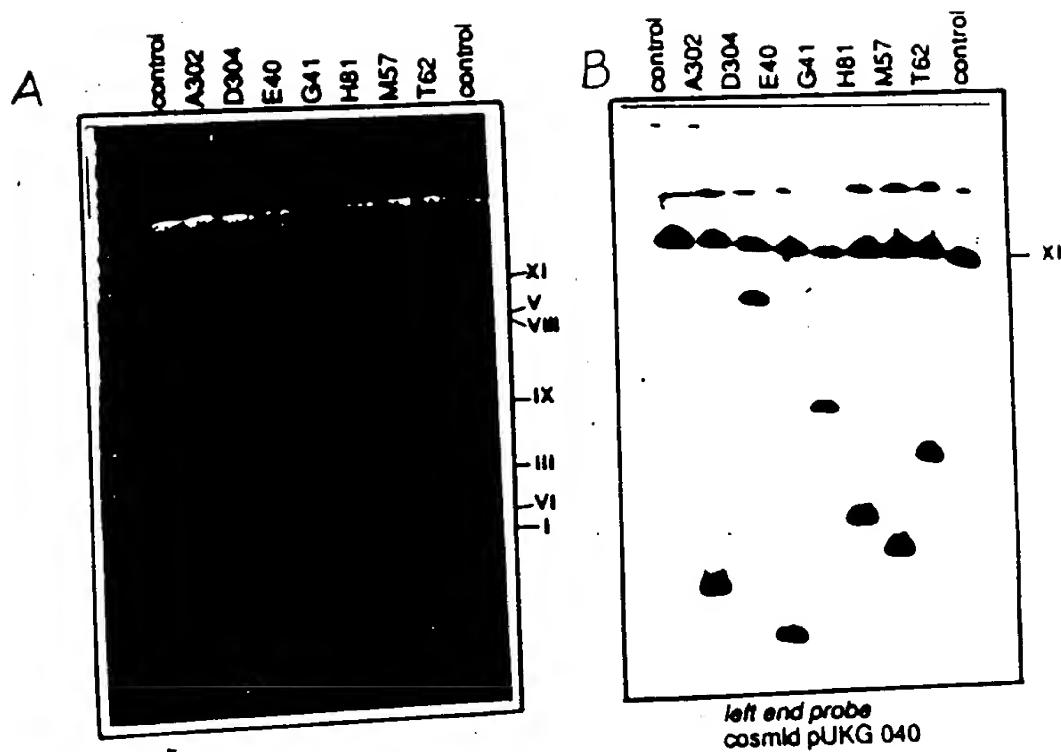
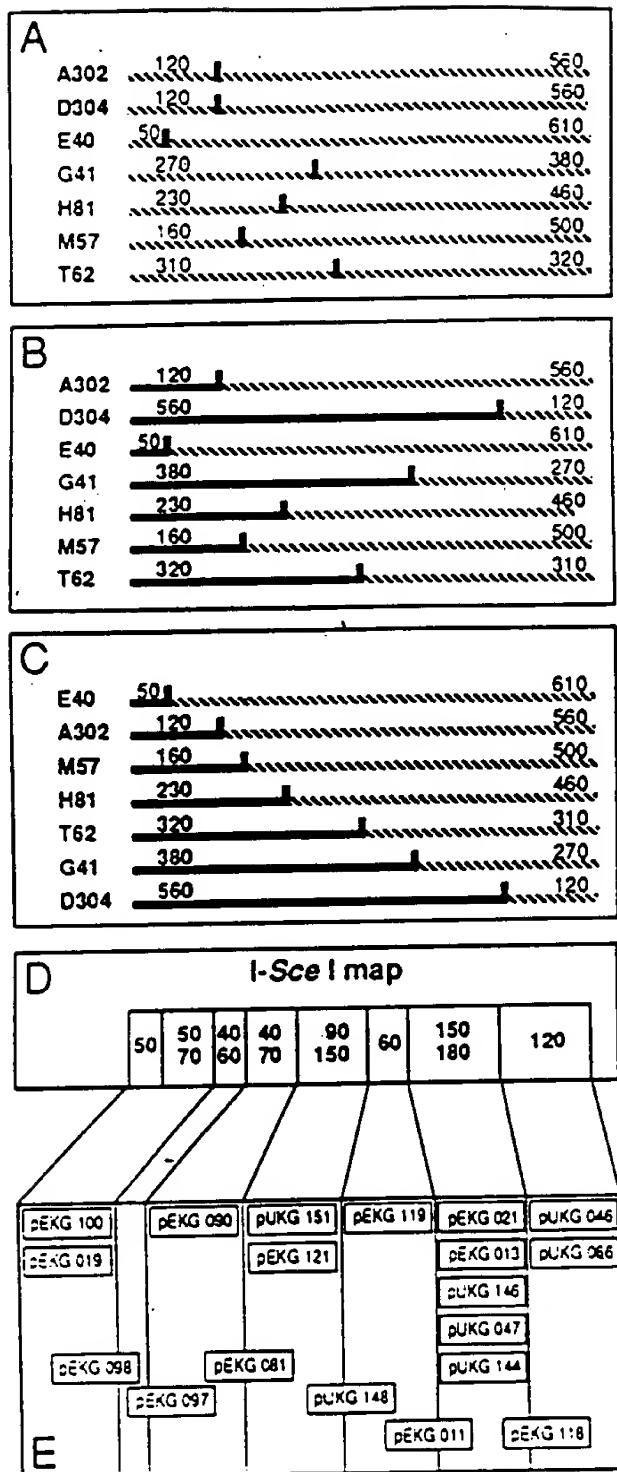


FIGURE 15



00222597-00222600

FIGURE 16

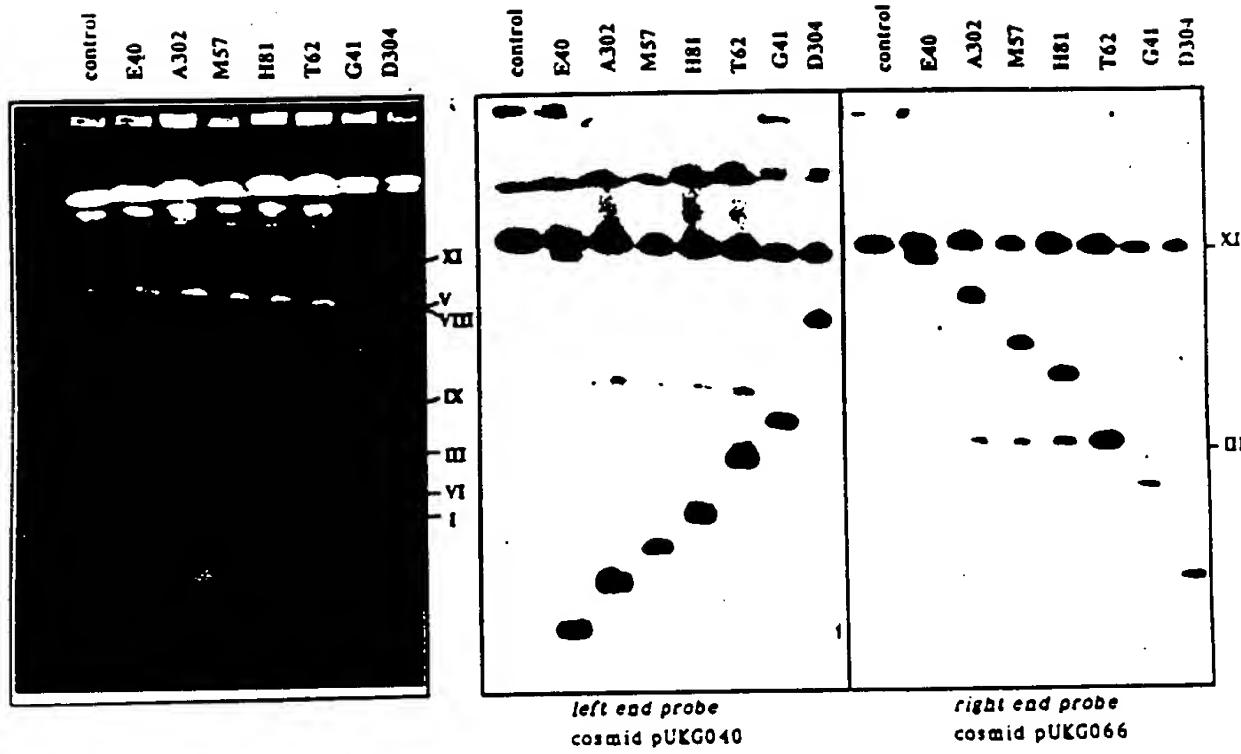
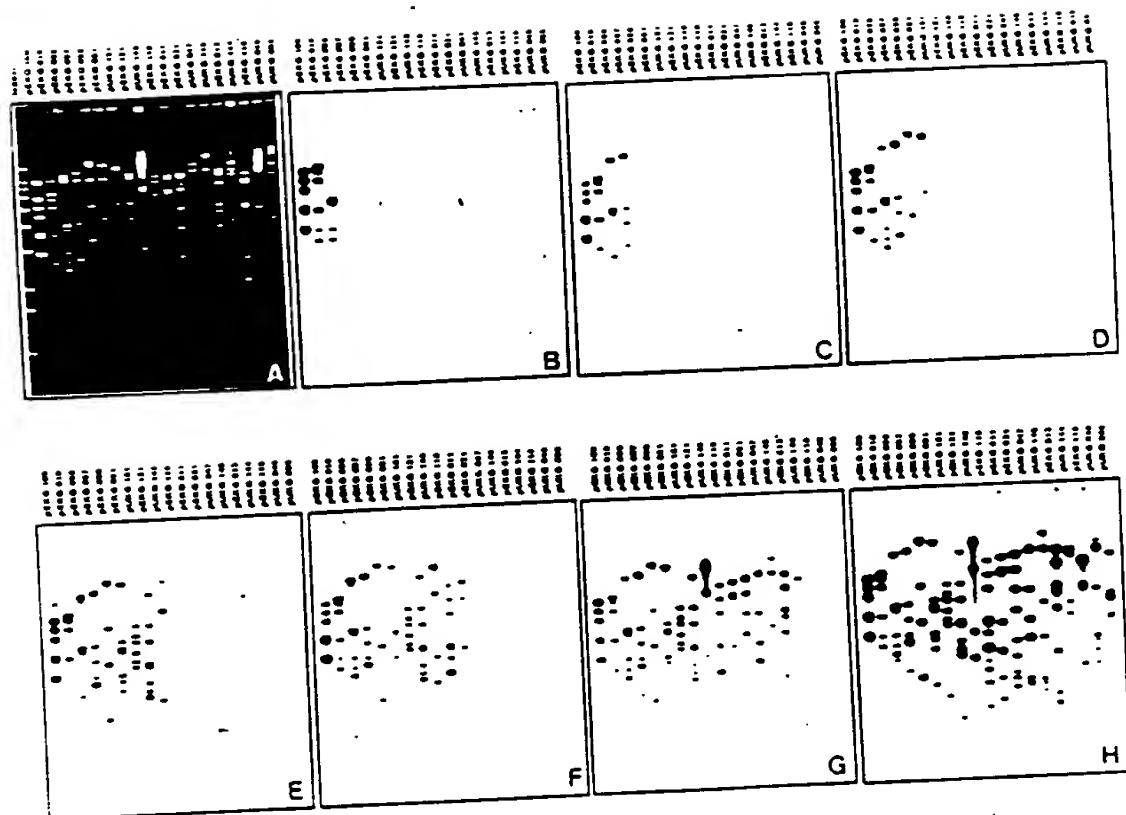


FIGURE 17



00014469 X - DATA FOR FIGURE 17

FIGURE 18

002270 26926400

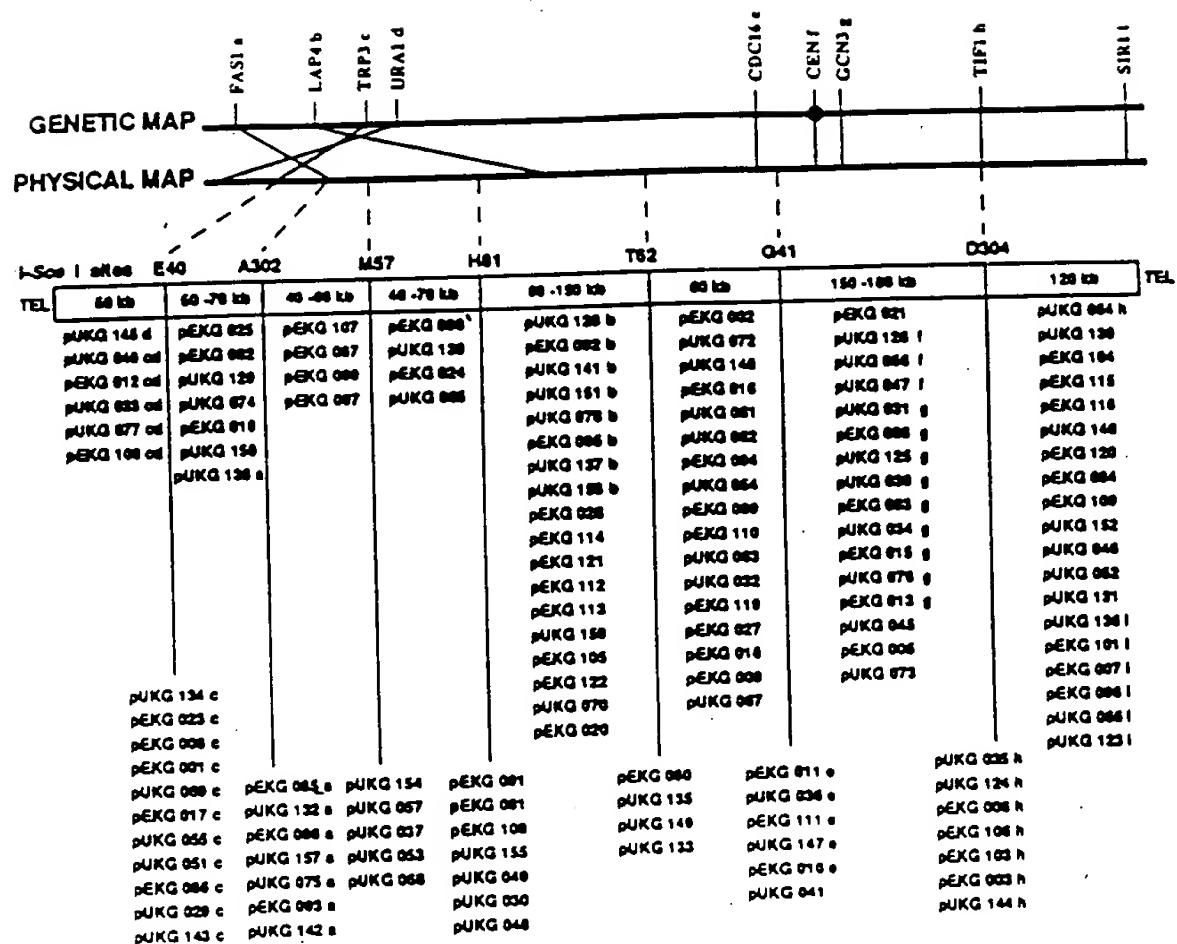


FIGURE 19

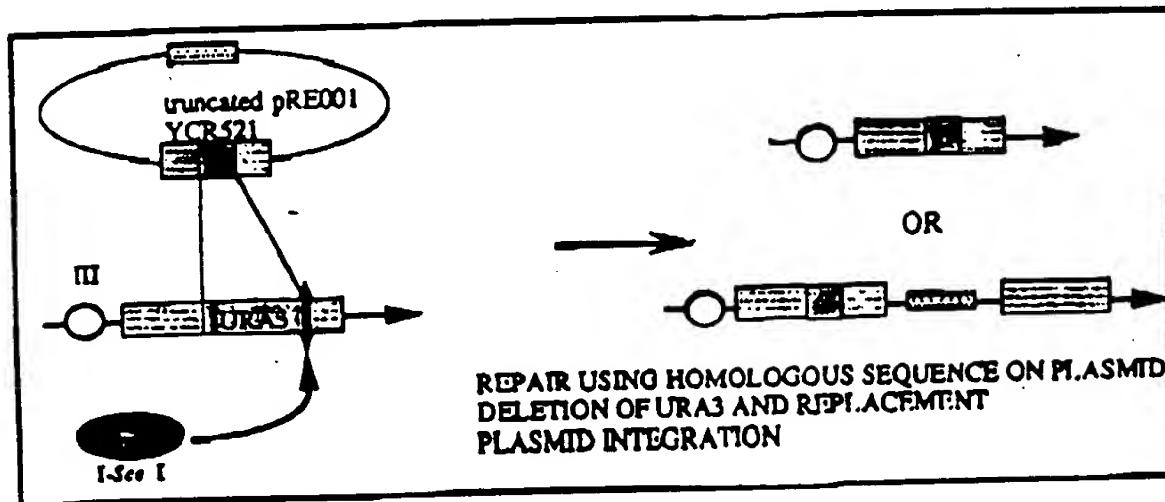
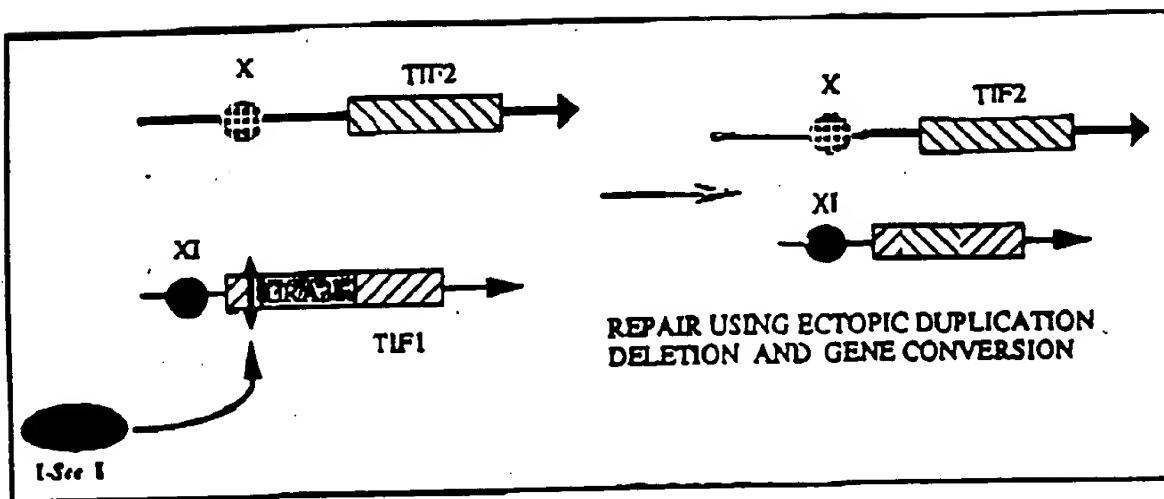


Figure 20

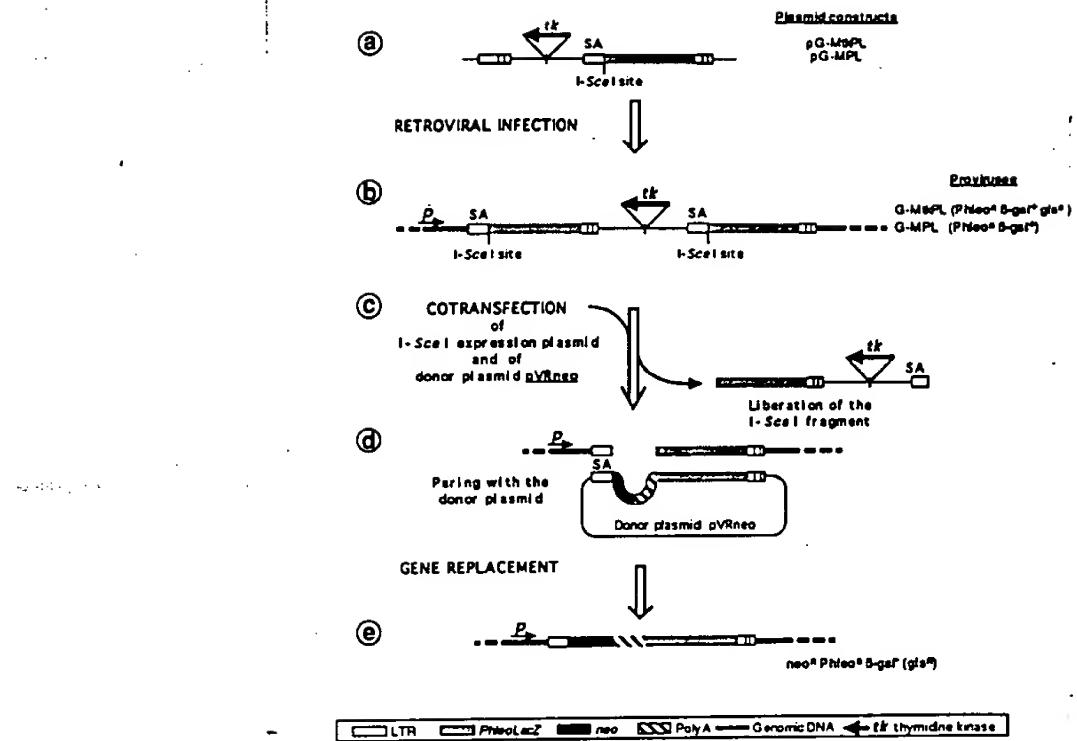


Figure 21

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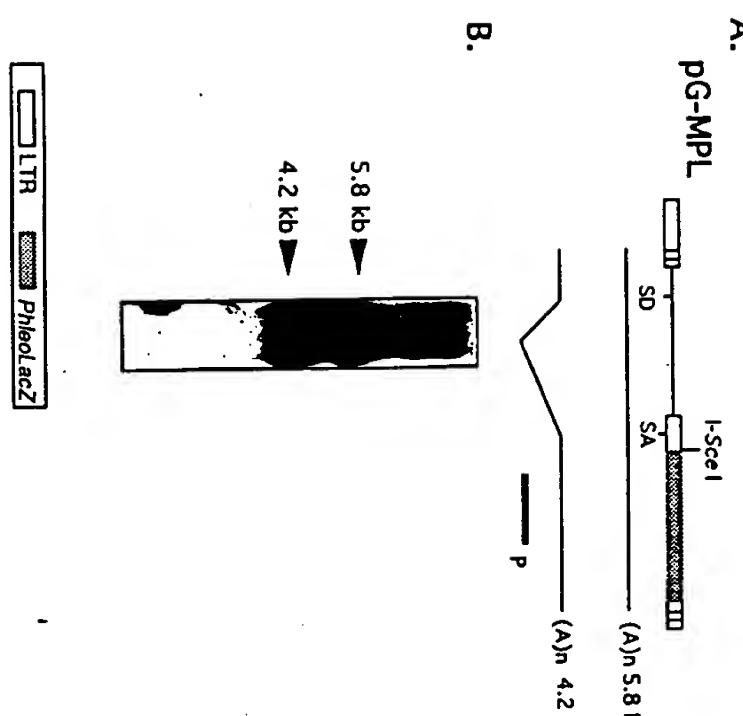
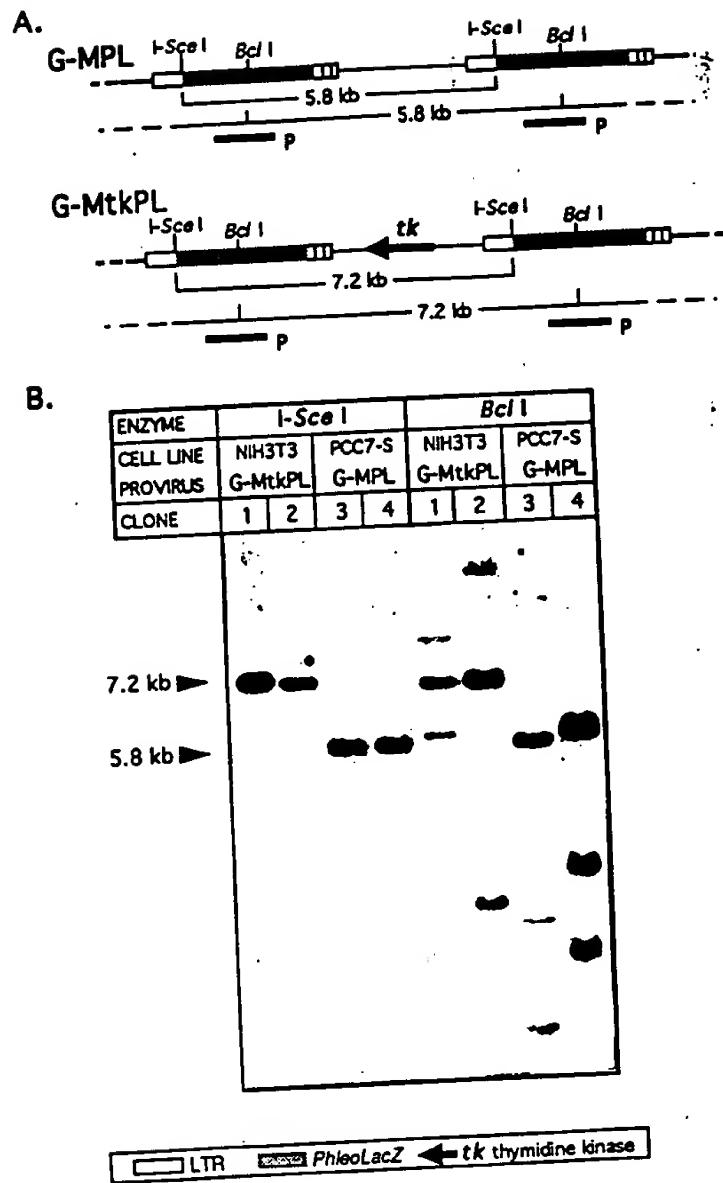
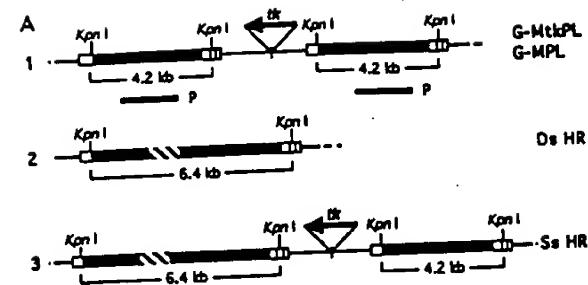


Figure 22

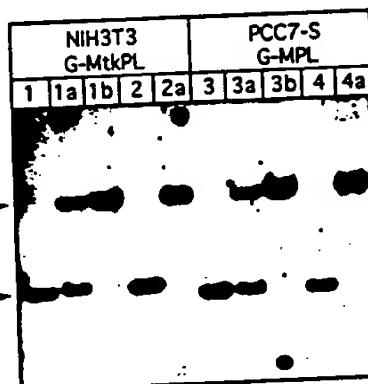


094528 X 69200

Figure 23



B



Legend: □ LTR   ■ PhoLacZ   ■ neo   ▨ PolyA   — Genomic DNA ← tK thymidine kinase

Figure 24

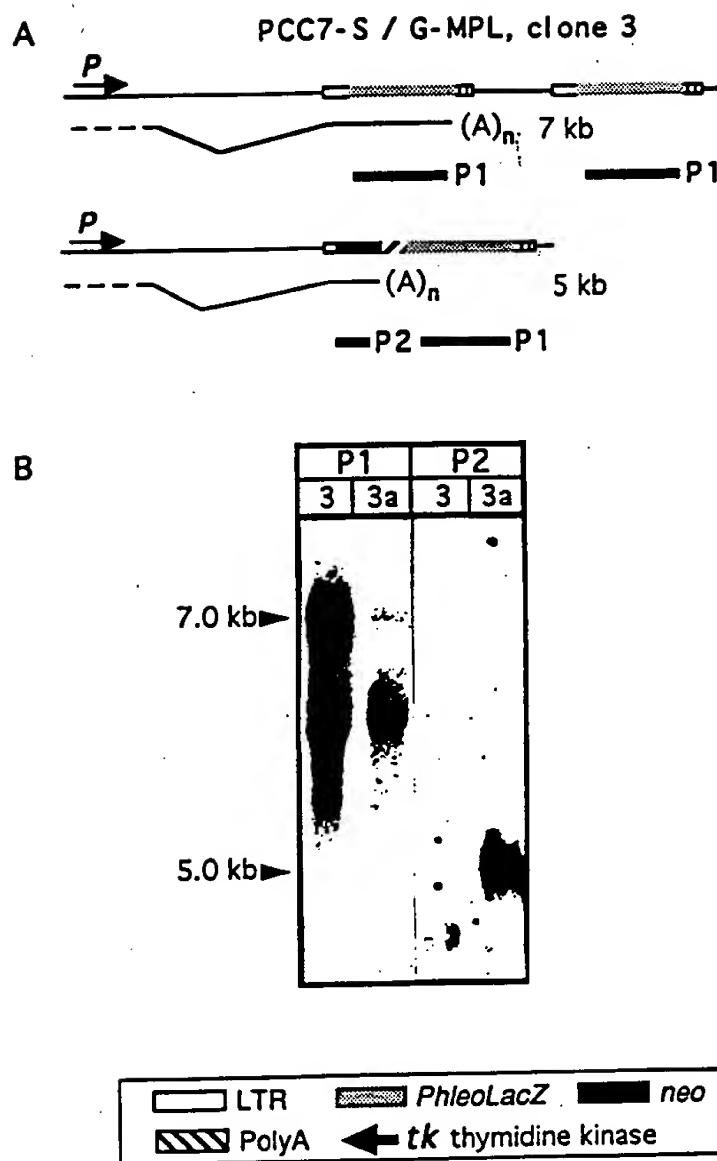


Figure 25

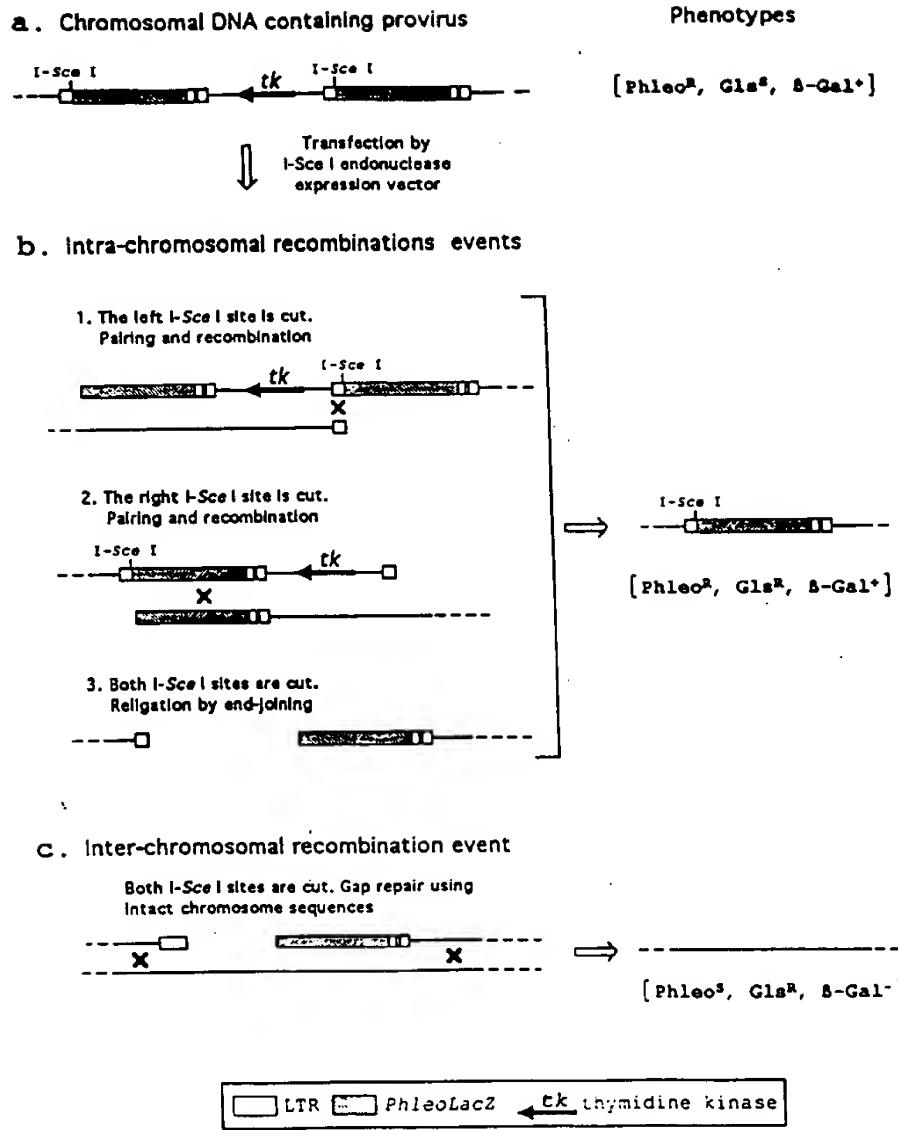
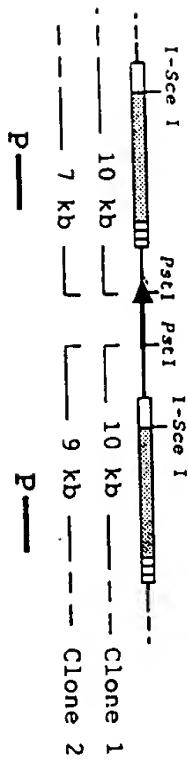


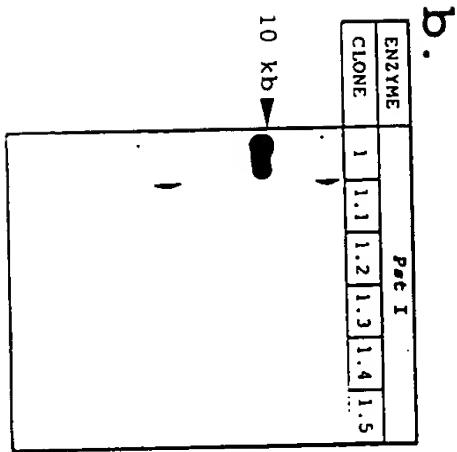
Figure 26

a. Parental DNA, G-MtkPL



b.

ENZYME	<i>Pst I</i>				
CLONE	1	1.1	1.2	1.3	1.4
					1.5



c.

ENZYME	<i>Pst I</i>					
CLONE	2	2.1	2.2	2.3	2.4	2.5
						2.6

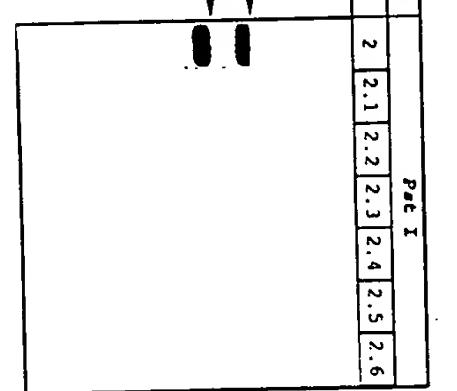
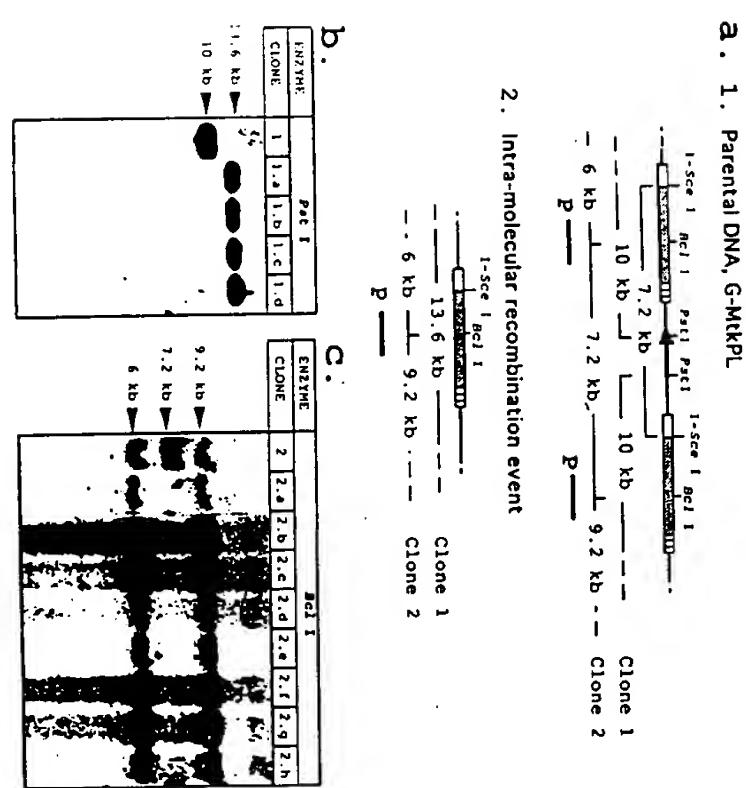


Figure 27

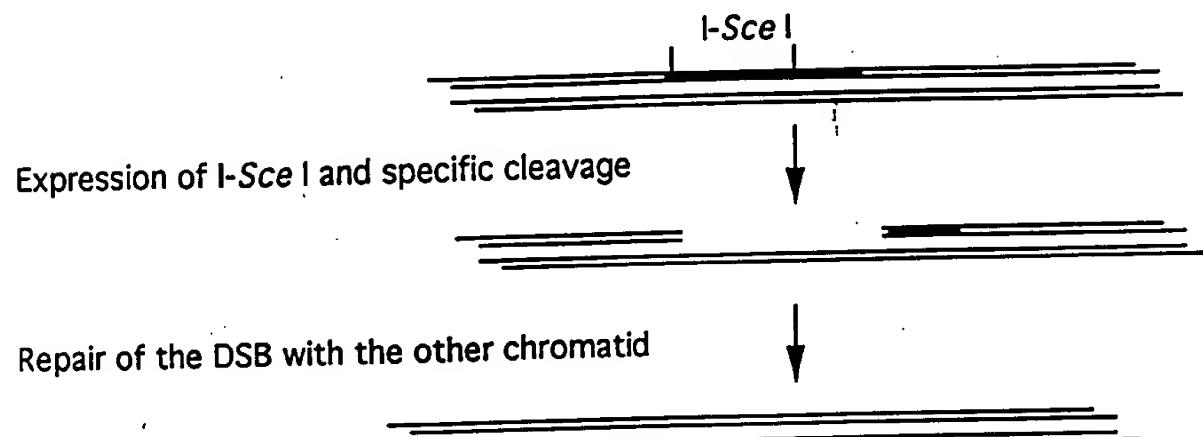


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Figure 28

## LOSS OF HETEROZYGOSITY

Integration of artificial site or presence of specific site



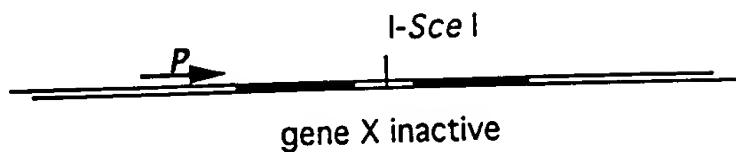
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CONFIDENTIAL

Figure 29

### CONDITIONNAL ACTIVATION (Tandem repeat)

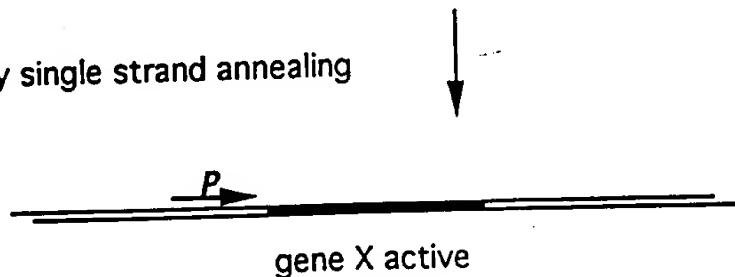
Integration of artificial site between tandem repeats



Expression of I-Sce I and specific cleavage



Repair of the DSB by single strand annealing

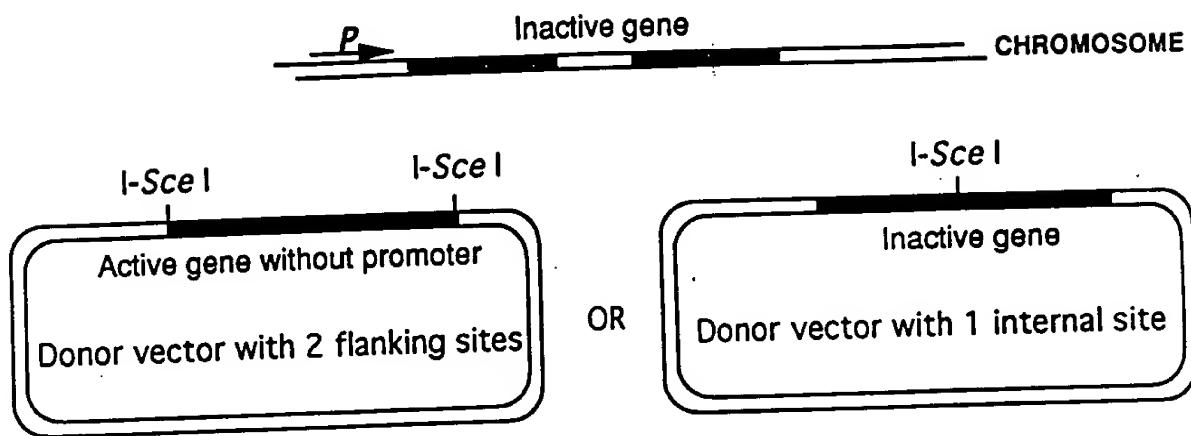


gene X active

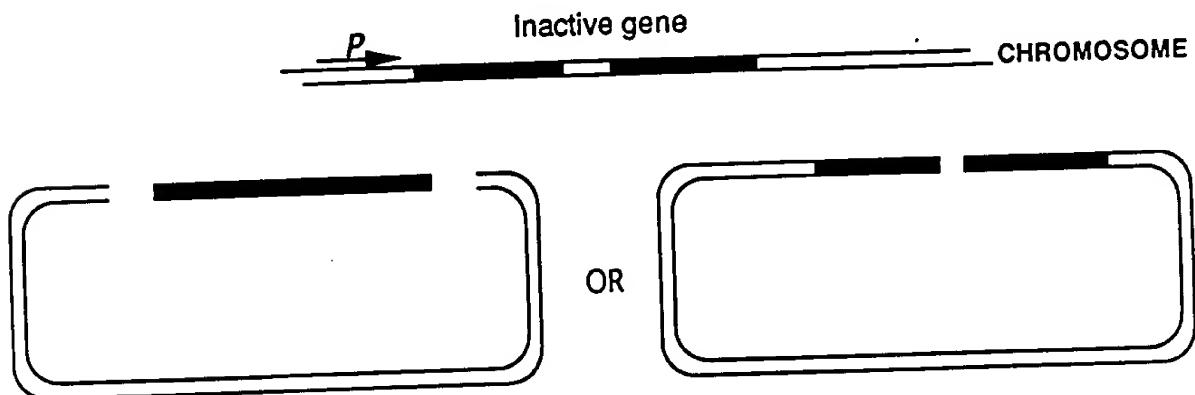
Figure 30

# ONE STEP REARRANGEMENT

Integration of artificial site or presence of specific site



Expression of I-Sce I enzyme  
and  
specific cleavage of the donor plasmid



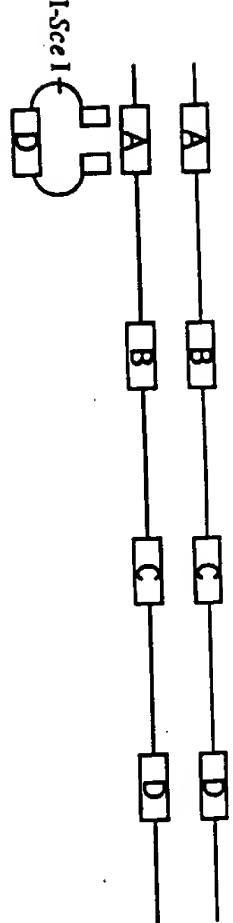
Recombination between the chromosome and plasmid



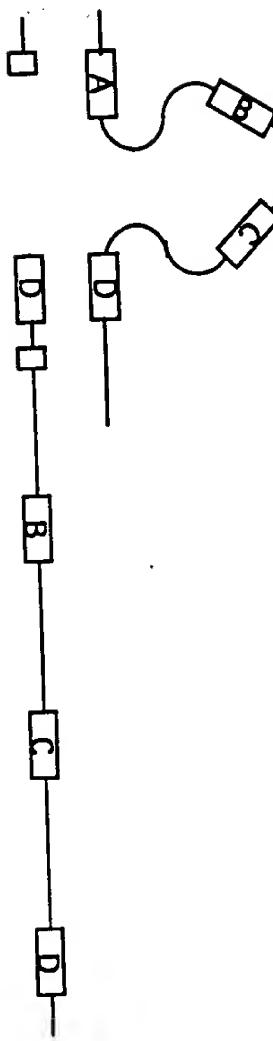
002000-2000000000

## DUPPLICATION OF A LOCUS

1 insertion of I-Sce I site by classical gene replacement



2 Specific cleavage by I-Sce I enzyme  
and repair of the break by homologous sequences



3 Duplication of the totality of the locus

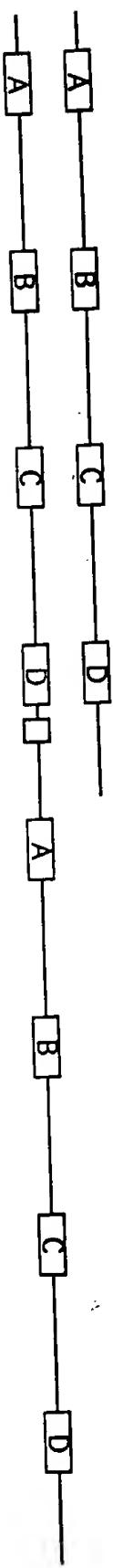
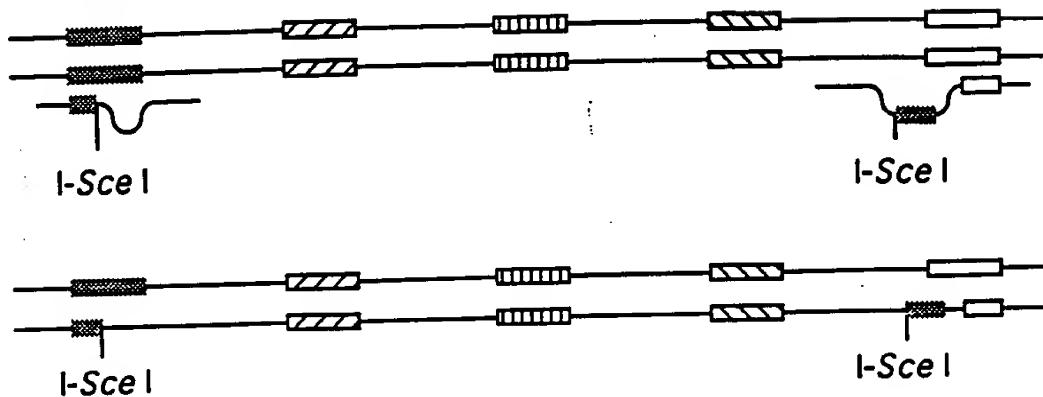


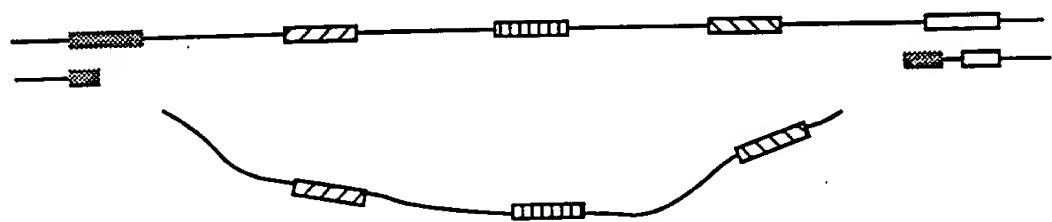
Figure 32

## DELETION OF A LOCUS

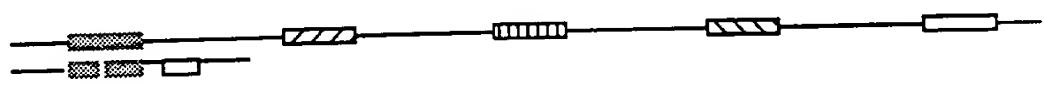
### 1 Insertion of two I-Sce I sites flanking the locus



### 2 Expression of the enzyme and cleavage



### 3 Recombination between the two ends



### 4 deletion of the locus



REPRODUCED BY OLYMPUS

Figure 33

